Some Primary Considerations in the Interpretation of the Dominant-Lethal Assay

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Introduction

Among the various procedures proposed for use in assessing the mutagenic potential of drugs, the dominant-lethal (D-L) assay stands currently as one of the few tests for measuring mutagenic effects on germ cells. Early identification of the D-L assay as a possible member of a test battery relates strongly to its being a mammalian model. Many scientists within the pharmaceutical industry believe that only those tests which utilize a mammalian model should be considered for primary use in drug safety evaluation protocols. The reason for such belief is obvious when one considers that the entire process of drug safety evaluation is oriented on established concepts in pharmacology and toxicology. Mammalian processes of assimilation, absorption, distribution, metabolism and elimination must be permitted to work on the chemical under test in order to provide some basis for extrapolating mutagenicity test data to man (*1*). Dose levels tested in these models and routes of drug administration should both reflect human use. Also, differences in the qualitative pharmacologic action of drugs must be considered as an essential part of the criteria applied to dose selection. Test reproducibility and dose–effect relationships must be emphasized in mutagenicity studies in order to identify those levels at which any mutagenic action is first detectable and to relate this level to the dose required for therapeutic efficacy.

In this presentation, data have been selected from a number of D-L studies which relate to these points and the difficulties encountered in interpreting D-L test results.

Methods

Random-bred CD-1 mice (Charles River), 8 weeks of age, were used in all experiments except where noted. Generally, 15 males were assigned to each test and control group, and 2 females were caged with each male. Pregnant females were identified by the presence of a mating plug. The number of total and dead implants/pregnant female were determined by autopsy at 12–14 days of pregnancy. Statistical analyses were computerized and all tests of significance were performed on arcsine transformed data. Weekly summations of test data were compared to a control regression computed across the entire 8 weeks of testing (*2*). These statistical models are discussed in the paper by Dr. David Salsburg (*3*).

Results and Discussion

Strain Characterization

Continuous surveillance of the mouse

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Table 1. Dominant-lethal assay: historical control, CD–1 strain, March 1971 through March 1973.

Week	Number pregnant	Embryos	Dead implants	Total implants	Tot. impl. preg. fem.	Dead impl. preg. fem.	Live impl. preg. fem.	% Dead impl.
1	871	9,880	745	10,625	12.20	0.86	11.34	7.0
2	1,009	11,499	884	12,383	12.27	0.88	11.40	7.1
3	966	11,228	823	12,051	12.48	0.85	11.62	6.8
4	901	10,360	830	11,190	12.42	0.92	11.50	7.4
5	863	10,100	749	10,849	12.57	0.87	11.70	6.9
6	745	8,869	667	9,536	12.80	0.90	11.90	7.0
7	718	8,463	631	9,094	12.67	0.88	11.79	6.9
8	747	8,680	703	9,383	12.56	0.94	11.62	7.5

strain selected for use in the D-L assay is an absolute necessity. Spurious increases in the number of dead implants/pregnant female, or a reduction in the total implants/pregnant female of the control group can have such a marked effect on determinations of dominant lethality that both these parameters must be monitored continuously. Strains that have high levels of fetal wastage due to genetic factors or infectious disease burdens are not well suited to use in the D-L assay.

Control data on 6820 pregnant females (CD-1 strain) are presented in Table 1. All data are expressed as a function of the week of mating following treatment of the male. The control males mated with these females had received physiological saline. This strain has consistently maintained an average level of total implants/pregnant female close to 12.50. The average number of dead implants/pregnant female is 0.89 and the average of living implants/pregnant female is 11.61. When the number of dead implants is compared to the total implants an average value of 7.1% is obtained.

An example of a shift in the reproductive behavior of this strain is shown in Table 2. During the period of November 1, 1972 to March 1, 1973 the number of dead implants/pregnant female rose to a value of 1.02. This was accompanied by a reduction in the number of living implants/pregnant female to 11.43. Total implants/pregnant female was 12.38 and the percent dead implants/total implants was 8.2. Although such a shift may appear slight, this degree of fetal wastage can produce problems in the interpretation of test results and reduce the sensitivity of the test (*4*). The rapid rise observed in this period suggests the introduction of an infectious disease entity although no overt clinical disease was evident.

Occasionally, a genetically aberrant male is encountered which produces a D-L effect in several stages of spermatogenesis. Table 3 shows such a result with significant responses in weeks 1 through 7. The compound involved normally produces a D-L effect in weeks 5 and 6. Additional analyses revealed a single male had produced this response.

Test Reproducibility

A true mutagenic response in the D-L assay can be characterized by a statistically significant increase in dead implants/pregnant female accompanied by a statistically significant reduction in living implants/pregnant female. Additionally, the compound involved should show a dose response relationship during a specific stage in the spermatogenic cycle. If a statistically significant response cannot be demonstrated reproducibly in the same stage of spermatogenesis, then a spurious positive result should be suspected. Table 4 demonstrates the typical response of the mutagen, ethyl methanesulfonate. In both experiments, the number of dead implants/pregnant female increases markedly during the first two weeks of mating. It should be noted that a significant decrease in the number of living implants per pregnant female occurs in the same two

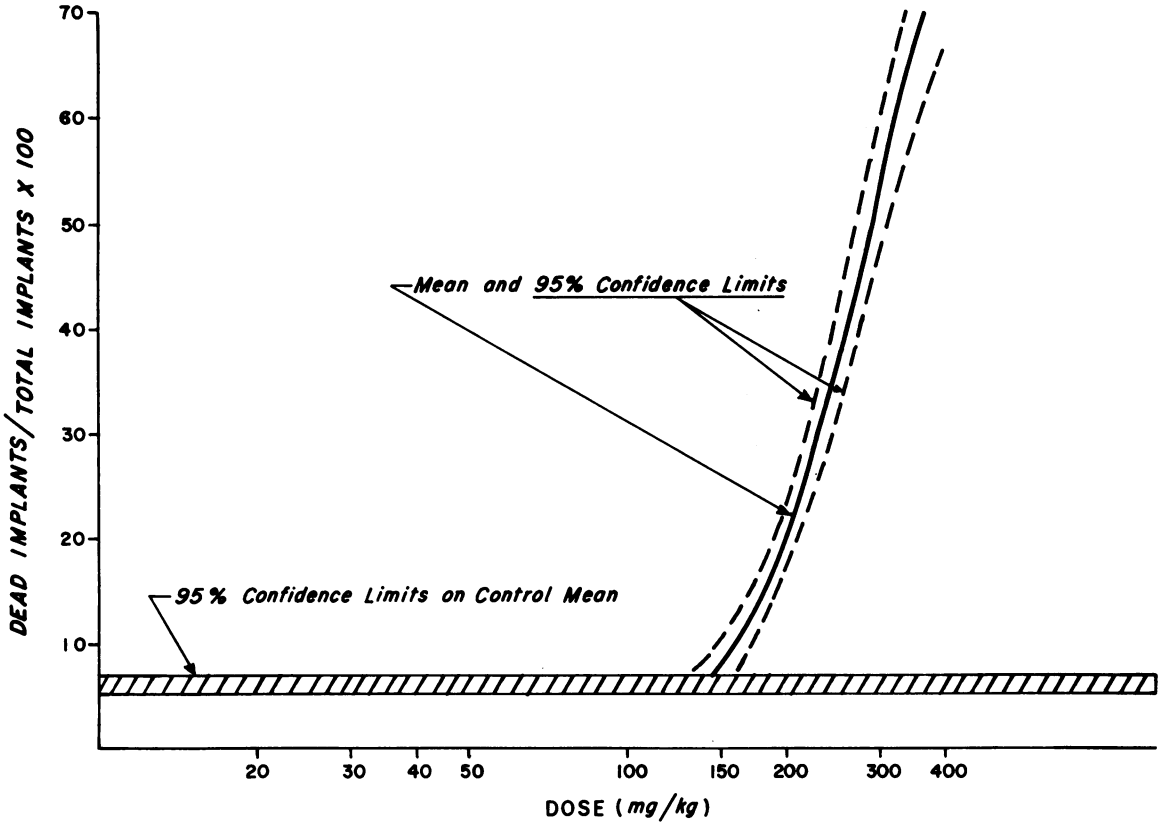

FIGURE 1. EMS dose response: days 7 through 11 post-injection.

weeks. A dose–response curve is shown in Figure 1 for the period 7–11 days following mating (*5*).

Another example of a reproducible D-L effect is depicted in Table 5. The purine analog, 6-mercaptopurine has produced a consistent D-L effect during weeks 5 and 6 of the spermatogenic cycle (*6*). Again, the parameter of living implants/pregnant female showed a simultaneous and significant reduction.

Nonreproducible Results

In contrast to the reproducibility obtained

Table 2. Dominant-lethal assay: historical control, CD–1 strain, November 1, 1972—March 1, 1973.

Week	Number pregnant	Embryos	Dead implants	Total implants	Tot. impl. / Preg. fem.	Dead impl. / Preg. fem.	Live impl. / Preg. fem.	% Dead impl.
1	148	1684	128	1812	12.24	0.86	11.38	7.06
2	163	1891	155	2046	12.55	0.95	11.60	7.58
3	166	1853	161	2014	12.13	0.97	11.16	7.99
4	156	1808	172	1980	12.69	1.10	11.59	8.69
5	156	1808	154	1962	12.58	0.99	11.59	7.85
6	129	1505	133	1638	12.70	1.03	11.67	8.12
7	122	1387	115	1502	12.31	0.94	11.37	7.66
8	98	1086	127	1213	12.38	1.30	11.08	10.47

Table 3. Results of testing with a genetically aberrant male.[a]

Week	Number pregnant		Total implants		Tot. impl. Preg. fem.		Dead impl. Preg. fem.		% Dead impl.		Live impl. Preg. fem.	
	C	T	C	T	C	T	C	T	C	T	C	T
1[b]	26	34	319	375	12.3	11.0	0.65	1.24	5.3	11.2	11.6	9.8
2[b]	43	46	535	586	12.4	12.7	1.19	1.32	9.5	10.4	11.3	11.4
3[b]	39	47	460	612	11.8	13.0	0.62	1.26	5.2	9.6	11.2	11.8
4[b]	34	47	435	619	12.8	13.2	0.79	1.38	6.2	10.5	12.0	11.8
5[b]	41	47	498	594	12.1	12.6	1.17	1.79	9.6	14.1	11.0	10.9
6[b]	28	29	360	338	12.9	11.7	1.07	2.28	8.3	19.5	11.8	9.4
7[b]	44	41	563	516	12.8	12.6	1.11	1.63	8.7	13.0	11.7	11.0
8	48	27	623	343	13.0	12.7	0.97	1.41	7.5	11.1	12.0	11.3

[a] C denotes controls; T denotes treated animals.
[b] Significance at the 1% level (dead implants/pregnant females).

during the same stage of spermatogenesis with a true mutagen, spurious or false-positive results do not repeat during the same stage of spermatogenesis. An example of this kind of results is shown in Table 6. The compound produced effects on two separate stages of spermatogenesis in the first two experiments. A third experiment performed at the same dose level was negative in both weeks 2 and 4. It should be noted that the parameter of living implants/pregnant female was not significantly reduced.

Tables 7–12 show the kind of inconsistencies which may occur in the dominant-lethal assay with a nonmutagenic substance. The response at week 7 at a dose of 7.5 mg/kg (Table 8) was not reproduced at a level of 75 mg/kg (Table 10). Further, the response

Table 4. Example of a reproducible result in the dominant-lethal assay with ethyl methanesulfonate, 300 mg/kg, oral.

Week	Number pregnant		Total implants		Tot. impl. Preg. fem.		Dead impl. Preg. fem.		% Dead impl.		Live impl. Preg. fem.	
	C	T	C	T	C	T	C	T	C	T	C	T
1[a]	58	43	704	488	12.14	11.35	1.12	1.81	9.23	15.98	11.02	9.53
2[a]	51	54	657	588	12.88	10.89	1.06	3.26	8 22	29.93	11.82	7.63
3	56	36	763	457	13.62	12.69	1.09	0.86	7.99	6.78	12.54	11.83
4	28	37	379	468	13.54	12.65	0.90	0.89	7.12	7.05	12.57	11.76
5	34	29	496	385	14.59	13.28	1.41	0.76	9.68	5.71	13.18	12.52
6	40	33	579	475	14.48	14.39	1.30	0.97	8.98	6.74	13.17	13.42
7	41	25	550	314	13.41	12.56	0.76	0.76	5.64	6.05	12.66	11.80
8	31	37	427	455	13.77	12.30	1.26	0.95	9.13	7.69	12.52	11.35
1[a]	32	42	379	473	11.84	11.26	0.84	2.86	7.12	25.37	11.00	8.40
2[a]	40	26	481	312	12.02	12.00	1.30	3.23	10.81	26.92	10.73	8.77
3	46	43	576	511	12.52	11.88	1.13	1.07	9.03	9.00	11.39	10.81
4	51	38	648	491	12.71	12.92	0.92	0.76	7.25	5.91	11.78	12.16
5	39	27	510	353	13.08	13.07	1.05	0.96	8.04	7.37	12.03	12.11
6	37	14	506	199	13.68	14.21	1.11	1.14	8.10	8.04	12.57	13.07
7	33	16	464	224	14.06	14.00	1.12	0.81	7.97	5.80	12.94	13.19
8	28	20	358	274	12.79	13.70	0.96	1.05	7.54	7.66	11.82	12.65

[a] Significance at the 1% level (dead implants/pregnant female).

Table 5. Example of a reproducible result in the dominant-lethal assay with 6-mercaptopurine, 150 mg/kg, IP.

Week	Number pregnant		Total implants		Tot. impl. Preg. fem.		Dead impl. Preg. fem.		% Dead impl.		Live impl. Preg. fem.	
	C	T	C	T	C	T	C	T	C	T	C	T
1	26	33	319	411	12.3	12.5	0.65	0.67	5.3	5.4	11.6	11.8
2	43	38	535	470	12.4	12.4	1.19	1.05	9.5	8.5	11.3	11.3
3	39	43	460	553	11.8	12.9	0.62	0.79	5.2	6.2	11.2	12.1
4	34	52	435	665	12.8	12.8	0.79	0.96	6.2	7.5	12.0	11.8
5 [a]	41	34	498	408	12.1	12.0	1.17	2.24	9.6	18.6	11.0	9.8
6 [a]	28	24	360	284	12.9	11.8	1.07	2.50	8.3	21.1	11.8	9.3
7	44	28	563	329	12.8	11.8	1.11	0.89	8.7	7.6	11.7	10.9
8	48	37	623	465	13.0	12.6	0.97	1.14	7.5	9.0	12.0	11.4
1	41	42	528	511	12.9	12.2	0.71	0.98	5.5	8.0	12.2	11.2
2	35	36	438	452	12.5	12.6	0.74	1.00	5.9	8.0	11.8	11.6
3	45	50	577	635	12.8	12.7	0.82	0.44	6.4	3.5	12.0	12.3
4	44	48	557	606	12.7	12.6	0.89	0.65	7.0	5.1	11.8	12.0
5 [a]	40	40	536	487	13.4	12.2	0.83	2.10	6.2	17.3	12.6	10.1
6 [a]	38	49	500	610	13.2	12.4	0.97	1.73	7.4	13.9	12.2	10.7
7	43	41	560	526	13.0	12.8	0.49	0.78	3.8	6.1	12.5	12.0
8	42	38	542	500	12.9	13.2	0.62	0.89	4.8	6.8	12.3	12.3

[a] Significance at the 1% level (dead implants/pregnant female).

Table 6. Example of a nonreproducible result in the dominant-lethal assay, experiment 50, dose 12 mg/kg, oral.

Week	Number pregnant		Total implants		Tot. impl. Preg. fem.		Dead impl. Preg. fem.		% Dead impl.		Live impl. Preg. fem.	
	C	T	C	T	C	T	C	T	C	T	C	T
1	39	35	470	461	12.05	13.17	0.95	0.97	7.87	7.38	11.10	12.20
2 [a]	39	45	510	613	13.08	13.62	0.92	1.87	7.06	13.70	12.15	11.76
3	35	41	438	557	12.51	13.59	0.97	1.15	7.76	8.44	11.54	12.44
4	34	34	436	434	12.82	12.76	1.12	1.21	8.72	9.45	11.71	11.56
5	39	35	496	446	12.72	12.74	1.13	0.97	8.87	7.62	11.59	11.77
6	40	41	490	486	12.25	11.85	0.75	0.90	6.12	7.61	11.50	10.95
7	37	38	464	471	12.54	12.39	0.92	1.37	7.33	11.04	11.62	11.03
8	23	25	284	333	12.35	13.32	1.43	1.16	11.62	8.71	10.91	12.16
1	38	32	363	305	9.55	9.53	1.03	0.66	10.74	6.89	8.53	8.88
2	46	45	540	550	11.74	12.22	1.33	1.22	11.30	10.00	10.41	11.00
3	39	48	472	580	12.10	12.08	0.85	1.15	6.99	9.48	11.26	10.94
4 [a]	49	32	671	433	13.69	13.53	0.86	1.44	6.26	10.62	12.84	12.09
5	28	32	387	467	13.82	14.59	1.32	1.16	9.56	7.92	12.50	13.44
6	31	28	429	398	13.84	14.21	0.77	0.96	5.59	6.78	13.06	13.25
7	27	29	353	421	13.07	14.52	0.93	0.93	7.08	6.41	12.15	13.59
8	31	24	404	339	13.03	14.12	0.81	0.67	6.19	4.72	12.23	13.46
1	31	32	362	366	11.68	11.44	0.65	0.72	5.52	6.28	11.03	10.72
2	36	37	485	466	13.47	12.59	0.92	0.92	6.80	7.30	12.56	11.68
3	51	35	551	443	10.80	12.66	0.92	1.20	8.53	9.48	9.88	11.46
4	39	47	509	609	13.05	12.96	0.74	0.94	5.70	7.22	12.31	12.02
5	39	36	497	461	12.74	12.81	1.23	0.97	9.66	7.59	11.51	11.83

[a] Significance at the 1% level (dead implants/pregnant female).

during week 3 at the dose level of 75 mg/kg was not reproduced at 150 mg/kg (Table 12). It should be noted that here again, no significant reduction was observed in either the total or living implants/pregnant female at any dose level during the test. The investigator is thus not mislead when the statistical analysis is performed on a weekly basis and the parameters of total and living implants are examined simultaneously with dead implants/pregnant female. In addition, this compound was not active in any test for mutagenic potential including the host-mediated and *in vivo* cytogenetic assays.

Table 7. Experimental inconsistency, experiment 42, dose level 7.5 mg/kg, oral.

	Number pregnant		Total implants		Tot. impl. / Preg. fem.		Dead impl. / Preg. fem.		% Dead impl.		Total live impl.		Live impl. / Preg. fem.	
Week	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	44	32	514	383	11.68	11.97	0.98	0.88	8.37	7.31	471	355	10.70	11.09
2	41	46	508	576	12.39	12.52	0.95	0.85	7.68	6.77	469	537	11.44	11.67
3	48	50	595	611	12.40	12.22	1.15	1.12	9.24	9.17	540	555	11.25	11.10
4	45	39	600	499	13.33	12.79	1.18	1.00	8.83	7.82	547	460	12.16	11.79
5	46	38	624	545	13.57	14.34	1.13	1.00	8.33	6.97	572	507	12.43	13.34
6	39	53	494	653	12.67	12.32	1.21	1.06	9.51	8.58	447	597	11.46	11.26
7	39	42	515	542	13.21	12.90	0.72	1.57	5.44	12.18	487	476	12.49	11.33
8	44	41	572	543	13.00	13.24	1.16	1.17	8.92	8.84	521	495	11.84	12.07

Table 8. Significance levels, dose level 7.5 mg/kg.

	Significance level		
Week	Dead impl. / Preg. fem.	Live impl. / Preg. fem.	Tot. impl. / Preg. fem.
1	0.10+	0.10+	0.10+
2	0.10+	0.10+	0.10+
3	0.10+	0.10+	0.10+
4	0.10+	0.10+	0.10+
5	0.10+	0.10+	0.10+
6	0.10+	0.10	0.10
7	0.01 [a]	0.10	0.10+
8	0.10+	0.10+	0.10+

[a] Weeks with significance at the 1% level.

Table 9. Experimental inconsistency, experiment 42, dose level, 75 mg/kg, oral.

	Number pregnant		Total implants		Tot. impl. / Preg. fem.		Dead impl. / Preg. fem.		% Dead impl.		Total live impl.		Live impl. / Preg. fem.	
Week	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	44	39	514	487	11.68	12.49	0.98	0.67	8.37	5.34	471	461	10.70	11.82
2	41	43	508	571	12.39	13.28	0.95	1.00	7.68	7.53	469	528	11.44	12.28
3	48	39	595	501	12.40	12.85	1.15	1.46	9.24	11.38	540	444	11.25	11.38
4	45	42	600	539	13.33	12.83	1.18	1.17	8.83	9.09	547	490	12.16	11.67
5	46	35	624	466	13.57	13.31	1.13	1.37	8.33	10.30	572	418	12.43	11.94
6	39	48	494	606	12.67	12.62	1.21	0.85	9.51	6.77	447	565	11.46	11.77
7	39	46	515	608	13.21	13.22	0.72	1.09	5.44	8.22	487	558	12.49	12.13
8	44	37	572	483	13.00	13.05	1.16	0.78	8.92	6.00	521	454	11.84	12.27

Table 10. Significance levels, dose level 75 mg/kg.

Week	Significance levels		
	Dead impl. Preg. fem.	Live impl. Preg. fem.	Tot. impl. Preg. fem.
1	0.10+	0.10+	0.10+
2	0.10+	0.10+	0.10+
3	0.01 [a]	0.10+	0.10+
4	0.10+	0.10+	0.10+
5	0.05 [b]	0.10+	0.10+
6	0.10+	0.10+	0.10+
7	0.10+	0.10+	0.10+
8	0.10+	0.10+	0.10+

[a] Weeks with significance at the 1% level.
[b] Weeks with significance at the 5% level.

Table 11. Experimental inconsistency, experiment 42, dose level, 150 mg/kg, oral.

Week	Number pregnant		Total implants		Tot. impl. Preg. fem.		Dead impl. Preg. fem.		% Dead impl.		Total live impl.		Live impl. Preg. fem.	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	44	31	514	371	11.68	11.97	0.98	0.97	8.37	8.09	471	341	10.70	11.00
2	41	49	508	607	12.39	12.39	0.95	0.94	7.68	7.58	469	561	11.44	11.45
3	48	40	595	485	12.40	12.12	1.15	0.98	9.24	8.04	540	446	11.25	11.15
4	45	38	600	483	13.33	12.71	1.18	0.95	8.83	7.45	547	447	12.16	11.76
5	46	49	624	671	13.57	13.69	1.13	1.10	8.33	8.05	572	617	12.43	12.59
6	39	43	494	544	12.67	12.65	1.21	0.79	9.51	6.25	447	510	11.46	11.86
7	39	46	515	620	13.21	13.48	0.72	1.07	5.44	7.90	487	571	12.49	12.41
8	44	37	572	478	13.00	12.92	1.16	1.27	8.92	9.83	521	431	11.84	11.65

Table 12. Significance levels, dose level 150 mg/kg.

Week	Significance levels		
	Dead impl. Preg. fem.	Live impl. Preg. fem.	Tot. impl. Preg. fem.
1	0.10+	0.10+	0.10+
2	0.10+	0.10+	0.10+
3	0.10+	0.10+	0.10+
4	0.10+	0.10+	0.10+
5	0.10+	0.10+	0.10+
6	0.10+	0.10+	0.10+
7	0.10+	0.10+	0.10+
8	0.05 [a]	0.10+	0.10+

[a] Weeks with significance at 5% level.

Effect of Dose Level

The qualitative pharmacologic action of drugs must be considered when choosing dose levels for D-L experiments. Drugs such as anesthetics and tranquillizers have such pronounced pharmacologic activity that excessive dose levels can produce marked temperature reductions and an inability to mate for several days following a single administration. An example of this kind of overdosage is shown in Figure 2. Here, 10°C degree reductions in body temperature were

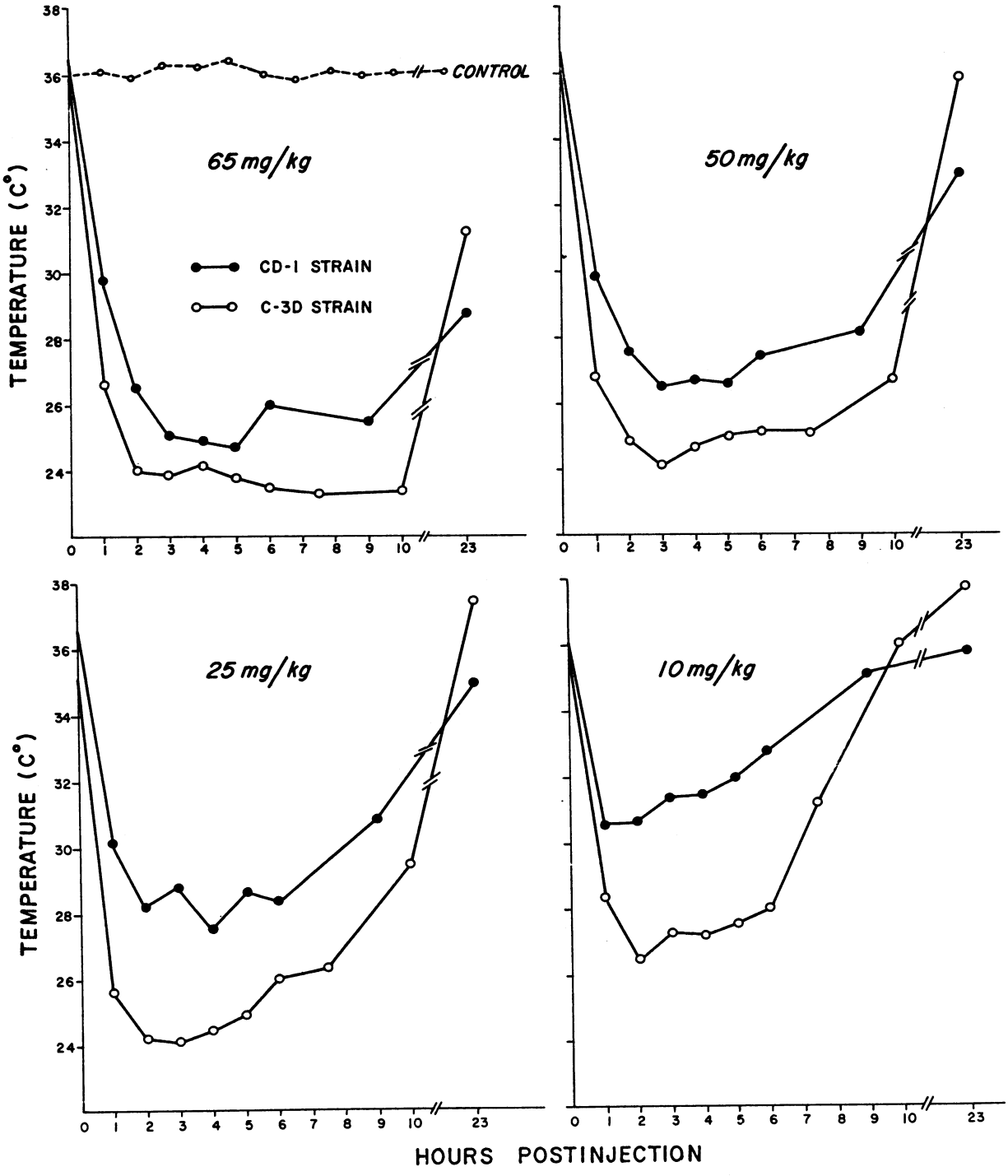

FIGURE 2. Effect of triflupromazine on body temperature.

observed at levels which were tested for mutagenic activity (*7*, *8*). Clearly, such reductions must reduce the overall metabolism of the test animal and therefore influence the metabolism of the drug. Levels of drug used in mutagenicity assessments should be chosen so as not to produce anorexia, sedation, or other exaggerated pharmacological effects (*9*).

Conclusions

In interpreting D-L data, the need for demonstrating a statistically significant and reproducible effect in the same stage of spermatogenesis cannot be over emphasized. In order to achieve consistent analyses, the degree of variability in important parameters of dead, living and total implants per pregnant female has to be firmly established for each strain of mouse employed. The statistical model utilized should include a transformation to reduce the effect of differing variances which occur in dead and total implants per pregnant female. Also, test results obtained during a specific stage of spermatogenesis should be compared to a control regression computed across the entire 8 weeks of testing. A dose response curve obtained during the active period of dominant lethality will provide additional evidence of compound activity. Data from D-L testing should be correlated and compared to other assessments of mutagenic potential such as the host-mediated and cytogenetic assays before applying the label of mutagen. Finally, the dosage regimen employed should not seriously alter the normal physiological processes of the test animal.

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